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Original Article

Role of miR-1 and its potential regulation of signaling pathways in bladder cancer: a comprehensive investigation based on TCGA, GEO, ArrayExpress, and bioinformatics analysis

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Abstract: Objective: To determine miR-1 expression and its potential molecular mechanism in bladder cancer. Methods: We analyzed the correlation between miR-1 expression and different clinicopathological features based on The Cancer Genome Atlas (TCGA). Gene Expression Omnibus (GEO) and ArrayExpress microarray datasets, as well as data from TCGA and the literature, were used to explore miR-1 expression in bladder cancer. A fixed-effects model and a random-effects model were used. We detected the diagnostic value of miR-1 in bladder cancer. Differentially expressed genes in TCGA, genes from predicting tools and upregulated genes in GSE24782 were then cross-referenced. The target genes from the literature were added, and the final genes were considered the promising target genes of miR-1 in bladder cancer. Subsequently, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were carried out. Results: The combined standardized mean difference was -1.29 (95% CI: -1.57, -2.00) and -1.83 (95% CI: -3.08, -0.58) based on the fixed- and random-effects models, respectively. The area under the curve was 0.9660 ($Q^* = 0.9135$) in the summarized receiver operating characteristic curve. The most significantly related GO annotation items were blood vessel development in biological processes, extracellular region in cellular components, and G-protein-activated inward-rectifier potassium channel activity in molecular functions. KEGG pathway analysis demonstrated that the promising target genes of miR-1 in bladder cancer were related to maturity onset diabetes of the young. Conclusion: The miR-1 was downregulated in bladder cancer. It might play an important role via its target genes in bladder cancer.

Keywords: MiR-1, bladder cancer, signaling pathways, TCGA, GEO

Introduction

Bladder cancer (BC), also called urothelial cancer, is the most frequent urinary tract malignancy and was estimated to cause 79,030 new cases and 16,870 deaths in 2017 worldwide [1-3]. BC has an intricate and multifactorial etiology, integrating genetics and the environment [4, 5]. The number of BC cases is rapidly increasing due to the increasing prevalence of tobacco use as well as other risk factors, such as variable industrial exposures [6-8]. Additionally, the risk of developing BC increases sharply with age, with 90% of cases diagnosed in people over 65 years of age. Moreover, the risk of BC for men is approximately three times higher than that for women [9]. BC can be categorized into two types according to the depth of invasiveness: non-muscle-invasive BC (NMIBC) and muscle-invasive BC. Nearly 75% of BC patients have NMIBC, which has a high recurrence rate of approximately 60% within five years and a relatively low progression rate (approximately 15%); similarly, patients with muscle-invasive BC experience a relatively high recurrence rate and an extremely high five-year mortality rate (approximately 50%-70%) [10-14]. Hence, there is a strong clinical need to identify and characterize novel markers for BC.

MicroRNAs (miRNAs), a class of small noncoding RNA molecules approximately 19 to 24

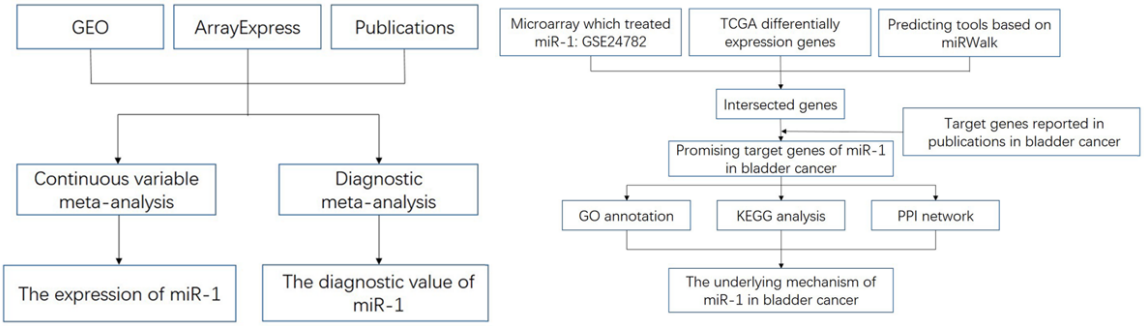

Figure 1. The design of our study.

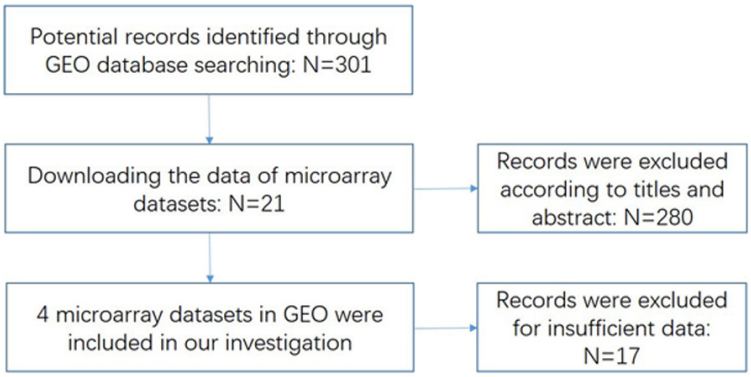

Figure 2. Flowchart of the selection of GEO microarray datasets.

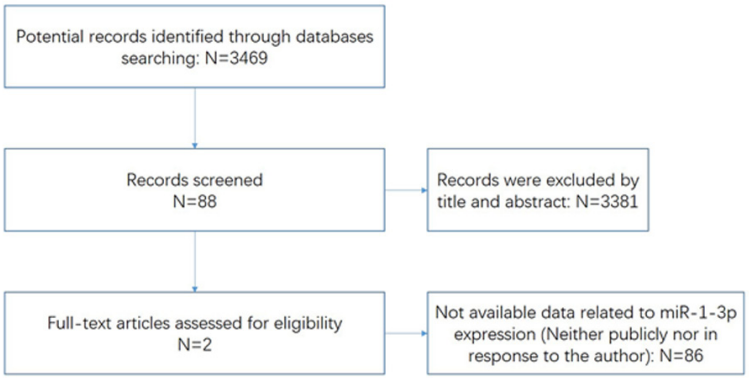

Figure 3. Flowchart of the selection of publications.

nucleotides in length, function in the posttranscriptional modulation of gene expression through RNA interference or gene silencing pathways. Numerous studies have shown that miRNAs play significant roles in the modulation of genes related to cancer metastasis and deterioration [15-19]. Several reports have also demonstrated that the expression levels of specific miRNAs in BC patients may be valuable markers for determining BC diagnosis and/or prognosis [20-25]. However, the clinical role and molecular mechanism of some miRNAs require further investigation.

Several studies have demonstrated that miR-1 is strongly associated with BC. MiR-1 is significantly downregulated in BC compared to adjacent tissues, suggesting that miR-1 may be used as a biomarker for prognostication and diagnosis as well as a therapeutic target for BC [26]; However, this conclusion is currently based on only a single study. Additionally, a tumor-suppressive function of miR-1 through the targeting of transgelin 2 (TAGLN2) in BC has been identified [27]. Moreover, miR-1 can suppress the proliferation, invasion, and migration of BC by enhancing the levels of secreted frizzled related protein 1 [28]. Although a series of studies of miR-1 in BC has been performed, there have been no systemic studies of the clinical value of miR-1 in BC, and the molecular mechanism contributing to the tumorigenesis of BC remains unclear.

Therefore, our study aims to determine the underlying molecular mechanism of BC and to explore innovative methods for its clinical diagnosis and treatment. This study investigates the clinical value of miR-1 in BC based on data

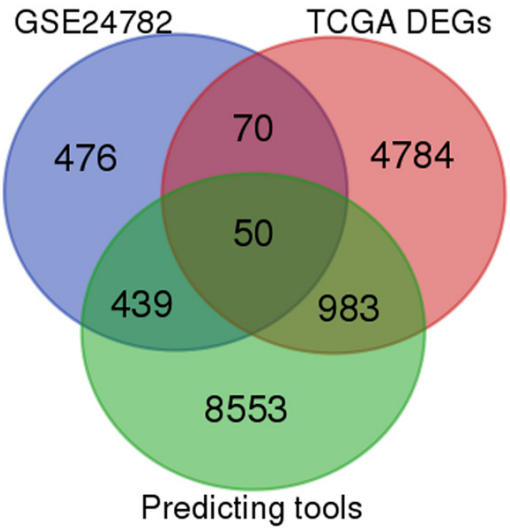

Figure 4. Venn diagram of the promising targets of miR-1 in bladder cancer.

from the Gene Expression Omnibus (GEO), ArrayExpress, and a meta-analysis of the literature. Additionally, a bioinformatics study was performed to predict target genes of miR-1, combining Gene Ontology (GO), pathway analysis, and network analysis, in order to further explore their underlying signaling pathways.

Materials and methods

Data collection from the GEO and ArrayExpress databases

Our study design is shown in **Figure 1**. We conducted data retrieval from the GEO and ArrayExpress databases. The search strategy used was as follows: (bladder OR urothelial) AND (cancer OR carcinoma OR tumor OR neoplas* OR malignan*). The restrictions in "entry type" and "organism" were "series" and "Homo sapiens", respectively. Microarray datasets that examined miR-1 expression in BC and nontumor tissues were included (**Figure 2**).

Additionally, to determine what role miR-1 might play in BC, we searched the GEO and ArrayExpress databases with the following keywords: (bladder OR urothelial) AND (cancer OR carcinoma OR tumor OR neoplas* OR malignan*) AND (miR-1 OR miRNA-1 OR microRNA-1 OR miR1 OR miRNA1 OR microRNA1 OR miR 1 OR miRNA 1 OR microRNA 1 OR miR-1-3p OR miRNA-1-3p OR microRNA-1-3p OR miR-1-1 OR miR-1-2 OR miR1-1 OR miR1-2). Datasets that interfered with miR-1 expression, via knockout or transfection, were further analyzed for miR-1-associated genes.

Retrieval of publications

We also searched PubMed, Science Direct, Google Scholar, Ovid, Wiley Online Library, EMBASE, Web of Science, Chong Qing VIP, CNKI, Wan Fang, and China Biology Medicine Disc with the following keywords: (bladder OR urothelial) AND (cancer OR carcinoma OR tumor OR neoplas* OR malignan*) AND (miR-1 OR miRNA-1 OR microRNA-1 OR miR 1 OR miRNA1 OR microRNA1 OR miR 1 OR miRNA 1 OR microRNA 1 OR miR-1-3p OR miRNA-1-3p OR microRNA-1-3p OR miR-1-1 OR miR-1-2 OR miR1-1 OR miR1-2). Studies that provided the mean, standard deviation, and case numbers of BC and nontumor groups, together with the microarray datasets from GEO and ArrayExpress, were included in our continuous variable meta-analysis (**Figure 3**). Additionally, publications for which true positive (TP), false positive (FP), false negative (FN), and true negative (TN) values could be calculated, together with the microarray datasets from GEO and ArrayExpress, were included in our diagnostic meta-analysis. We also collected the putative target genes in BC that were reported in the literature.

TCGA data mining

We downloaded a BC miRNA matrix from cBioPortal (http://www.cbioportal.org/index.do). Data were normalized to a log2 scale. The TCGA data were also included in our continuous variable meta-analysis and diagnostic meta-analysis. We downloaded mRNA data regarding the entry of colorectal cancer from The Cancer Genome Atlas (TCGA). The DESeq data R package was utilized to determine differentially expressed genes. Additionally, miR-1 expression data and its clinicopathological parameters were downloaded from TCGA.

Statistical analysis

All data from the microarray datasets and TCGA were normalized to a log2 scale. For miR-1 expression and its clinicopathological parameters from TCGA, independent-sample *t*-tests and paired sample *t*-tests were applied in SPSS 23 to analyze their association.

Table 1. MiR-1 expression and its clinicopathological parameters in bladder cancer from TCGA

Clinicopathological Feature		N	Has miR-1-2 relevant expression		
			M ± SD	T	P
Tissue	Noncancerous	19	7.7766 ± 0.8595	7.290	< 0.001
	Cancerous	19	4.3745 ± 1.9532		
Age	< 50	19	2.9715 ± 2.3626	2.957	0.003
	≥ 50	383	4.3678 ± 1.9906		
Gender	Male	298	4.2190 ± 2.1032	-1.454	0.147
	Female	105	4.5274 ± 1.7791		
Smoking	Past or current smoking	282	4.3630 ± 2.0726	1.021	0.308
	Never smoke	108	4.1294 ± 1.8824		
Subtype	Papillary	129	3.9144 ± 1.8381	-2.674	0.011
	Nonpapillary	270	4.4642 ± 2.0845		
Lymphovascular invasion	Yes	147	4.5264 ± 2.1146	2.204	0.008
	No	130	3.9749 ± 2.0364		
Pathologic stage	I-II	132	3.8159 ± 2.0336	3.402	0.001
	III-IV	269	4.5405 ± 1.9898		
Recurrence	Yes	69	4.7655 ± 1.7752	2.372	0.019
	No	224	4.1560 ± 2.1365		
Histologic grade	High grade	379	4.3506 ± 2.0194	2.231	0.026
	Low grade	21	3.3424 ± 1.9408		

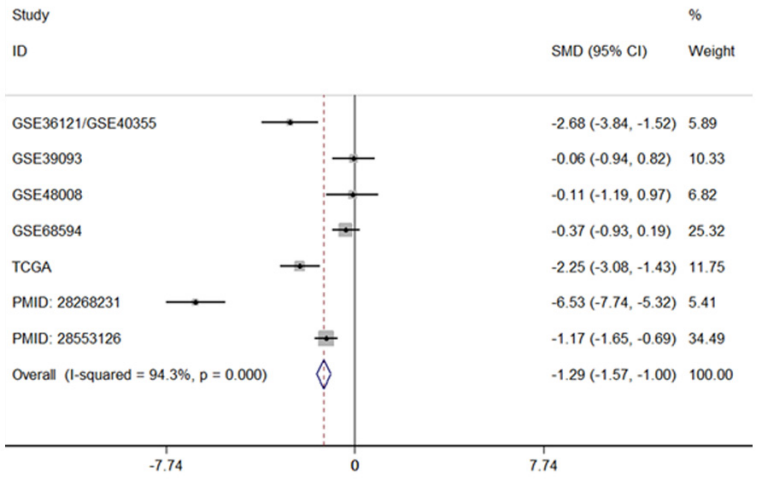

Figure 5. Forest plot of miR-1 expression in bladder cancer based on the fixed-effects model.

The mean, standard deviation, and number of cases in BC and nontumor groups were calculated based on all the studies included. Stata 14 was used to perform continuous variable meta-analysis. Initially, a fixed-effects model was chosen, and a random-effects model was then used when we found heterogeneity (I^2 > 50%). A funnel plot was constructed to test for publication bias. To determine the heterogeneity, we performed sensitivity analysis. After removing the most discordant studies, the forest plot was reconstructed.

For diagnostic tests, the TP, FP, FN, and TN values based on the individual studies included were calculated via SPSS 23. MetaDiSc 1.4 was used to perform the diagnostic meta-analysis. Threshold-effects analysis was performed to identify the source of heterogeneity. We also described the diagnostic value of miR-1 in BC using summarized receiver operating characteristic (SROC) curve analysis.

Identification of promising target genes of miR-1 in BC

In GSE24782, the downregulated genes with a log2FC < -1 were considered significant. In TCGA, the upregulated genes with a log2FC > 1 and P < 0.05 were considered significant. We also utilized the online prediction tool miRWalk 2.0 to predict the target genes of miR-1 using a computer algorithm. Microt4, miRWalk, miRanda, miRBridge, miRDB, miRMap, miRNAMap, PicTar, PITA, RNA22, RNAhybrid, and Targetscan were selected in miRWalk 2.0. Genes were selected that appeared at least twice among the 12 online prediction tools. The promising target genes of miR-1 were cross-referenced

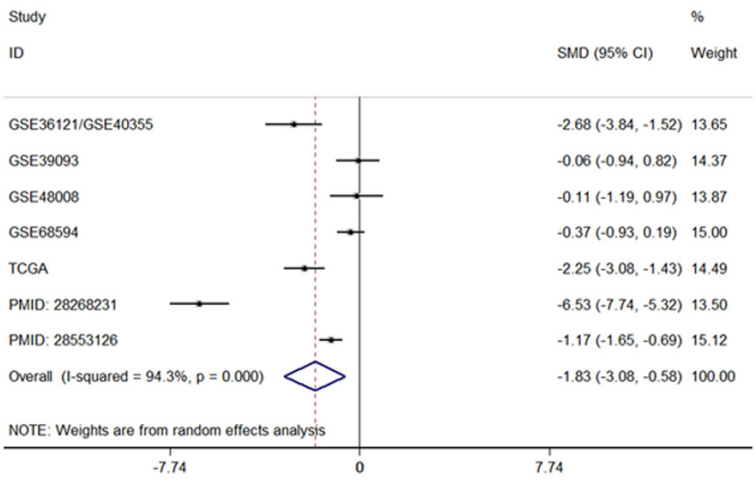

Figure 6. Forest plot of miR-1 expression in bladder cancer based on the random-effects model.

with differentially expressed genes among the GSE24782, TCGA, and selected genes in the prediction tools (**Figure 4**). Finally, the target genes in BC from relevant publications were added.

Pathway analysis in silico

To determine the underlying mechanism of miR-1 in BC, the promising target genes of miR-1 were used to perform GO annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis in DAVID 6.8 (https://david-d.ncifcrf.gov/) [29-33]. The GO annotation items included biological process (BP) and cellular component (CC) as well as molecular function (MF). A protein-protein interaction (PPI) network was constructed using STRING (http://www.string-db.org) [34-38] to characterize the link between possible target genes of miR-1. Disconnected nodes were hidden in the network.

Results

MiR-1 expression and its clinicopathological parameters in bladder cancer from TCGA

MiR-1 had lower expression in bladder cancer tissues compared to noncancerous tissues (4.3745 ± 1.9532 vs. 7.7766 ± 0.8595, respectively, P = 0.000). The miR-1 expression level was 4.3678 ± 1.9906 in patients over 50 years old and 2.9715 ± 2.3626 in patients under 50 years old (P = 0.003). Papillary BC had significantly lower miR-1 expression compared to nonpapillary BC (3.9144 ± 1.8381 vs. 4.4642 ± 2.0845, respectively, P = 0.011). Patients with lymphovascular invasion had higher miR-1 expression levels compared to those without (4.5264 ± 2.1146 vs. 3.9749 ± 2.0364, respectively, P = 0.008). The miR-1 expression level was higher in patients diagnosed with advanced pathologic stages (III-IV) than in those with lower pathologic stages (I-II) (4.5405 ± 1.9898 vs. 3.8159 ± 2.0336, respectively, P = 0.001). Patients who experienced recurrence of BC exhibited higher levels of miR-1 than those who did not (4.7655 ± 1.7752 vs. 4.1560 ± 2.1365, respectively, P = 0.019). The miR-1 level was 4.3506 ± 2.0194 in patients with high histologic tumor grades and 3.3424 ± 1.9408 in patients with low histologic grades (P = 0.026). The relationships between miR-1 expression and other clinicopathological parameters are shown in **Table 1**.

The expression of miR-1 in bladder cancer

Continuous variable meta-analysis showed lower miR-1 expression. Based on the fixed-effects model, the standardized mean difference (SMD) was -1.29 (95% CI: -1.57, -2.00; I^2 = 94.3%; **Figure 5**). For the random-effects model, the SMD was -1.83 (95% CI: -3.08, -0.58; I^2 = 94.3%; **Figure 6**). There was no evidence in either Begg's funnel plot or Egger's plot analysis that our study included publication bias (P > 0.05; **Figure 7**). Sensitivity analysis showed that GSE68594 and one publication [28] might be the sources of heterogeneity (**Figure 8**). After removal of GSE68594 and that publication, the combined SMD was -1.21 (95% CI: -1.55, -0.87; I^2 = 82.6%; **Figure 9**). Other publications that reported miR-1 expression in BC but could not be included in the meta-analysis are shown in **Table 2**.

Diagnostic value of miR-1 downregulation in bladder cancer

In diagnostic forest plots, the combined sensitivity, specificity, positive likelihood ratio, nega-

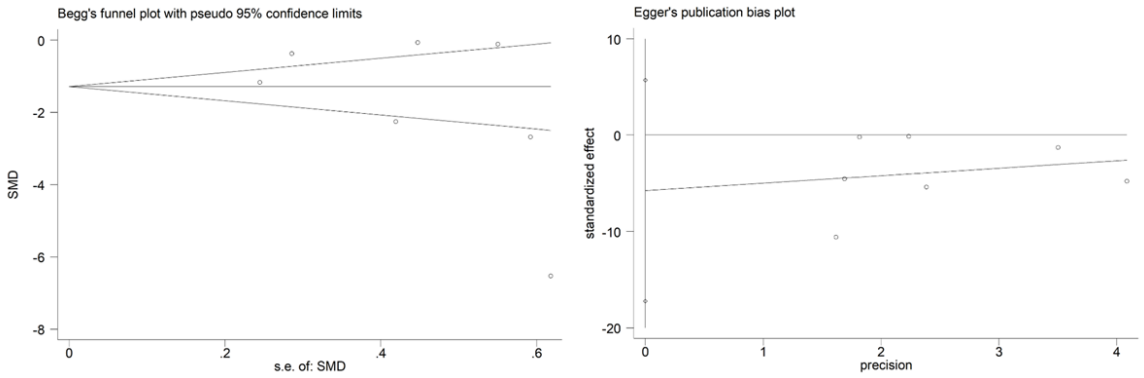

Figure 7. Begg's funnel plot and Egger's plot to test publication bias.

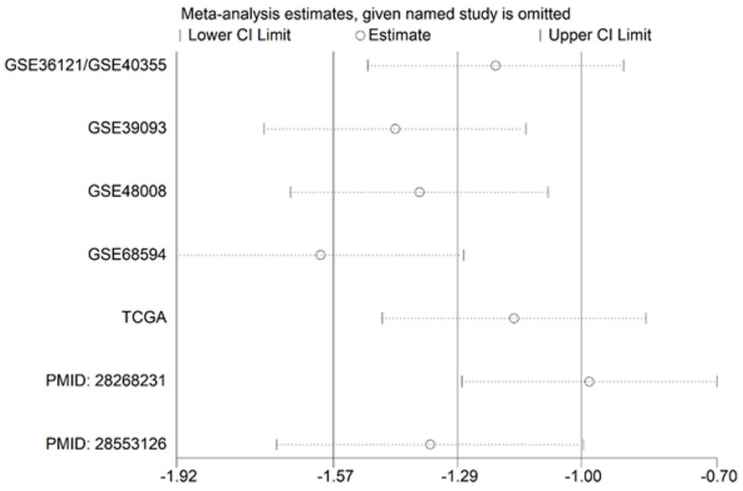

Figure 8. Sensitivity analysis.

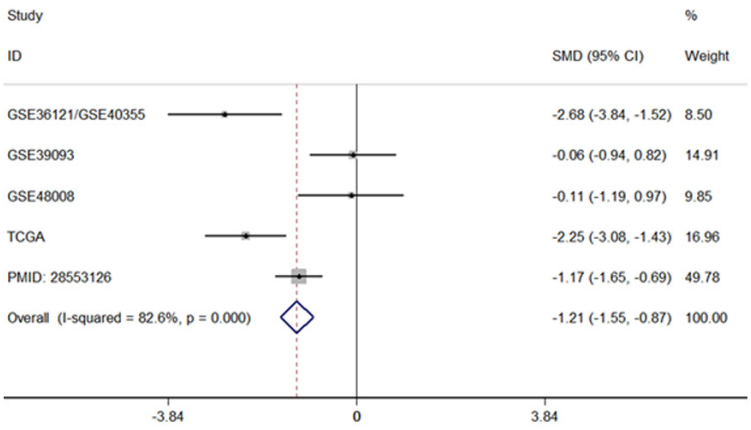

Figure 9. Forest plot after removal of GSE68594 and one publication (PMID: 28268231).

tive likelihood ratio, and odds ratio were 0.79 (95% CI: 0.71, 0.85), 0.86 (95% CI: 0.80, 0.91), 12.34 (95% CI: 1.49, 102.39), 0.14 (95% CI: 0.03, 0.66), and 88.03 (95% CI: 18.38, 421.52) (**Figure 10**). In the summarized receiver operating characteristic (SROC) curve, the area under the curve (AUC) was 0.9660 (Q* = 0.9135; **Figure 11**). We did not find a threshold effect in our study (P = 0.939).

Bioinformatics analysis based on promising target genes in bladder cancer

In total, 55 genes were considered promising target genes of miR-1. The most significantly related GO annotation items (**Figure 12**) were blood vessel development in biological processes, extracellular region in cellular components, and G-protein-activated inward-rectifier potassium channel activity in molecular functions. KEGG pathway analysis (**Figure 12**) demonstrated that the promising target genes of miR-1 in BC were related to maturity onset diabetes of the young. In the PPI network (**Figure 13**), there were 55 nodes and 10 edges with a PPI enrichment *p*-value of 0.118. TAT, FOXA2, CDX2, HNF4A, IFIT2, IFIT1, HIST1H2AC, KLK2, PTMA, SLC6A7, KCNJ10, KCNJ16, ELAVL2, and SRSF9 were highlighted in the PPI network.

Table 2. Studies of miR-1 expression not included in the continuous meta-analysis

First author	Title	The expression condition of miR-1	Citations
Wei Y	Comprehensive investigation of aberrant microRNA profiling in bladder cancer tissues	Downregulated	[25]
Song T	Differential miRNA expression profiles in bladder urothelial carcinomas	Downregulated	[39]
Wang T	Hsa-miR-1 downregulates long noncoding RNA urothelial cancer associated 1 in bladder cancer	Downregulated	[57]
Pignot G	MicroRNA expression profile in a large series of bladder tumors: identification of a 3-miRNA signature associated with aggressiveness of muscle-invasive bladder cancer	Downregulated	[58]
Zhao Y	MicroRNA response elements-regulated TRAIL expression shows specific survival-suppressing activity on bladder cancer	Downregulated	[42]
Wang W	MiR-1-3p inhibits the proliferation and invasion of bladder cancer cells by suppressing CCL2 expression	Downregulated	[43]
Itesako T	The microRNA expression signature of bladder cancer by deep sequencing: the functional significance of the miR-195/497 cluster	Downregulated	[59]
Yoshino H	The tumour-suppressive function of miR-1 and miR-133a targeting TAGLN2 in bladder cancer	Downregulated	[27]

Discussion

In this study, eligible published studies and data from microarray datasets and TCGA were analyzed to identify aberrant miR-1 expression in BC. The results showed that miR-1 was expressed at a lower level in BC cases compared to noncancerous cases and that miR-1 expression was also related to the development of BC, likely via influencing various key pathways.

To date, a growing body of evidence has suggested that miRNAs, such as miR-1, miR-133a, miR-133b, and miR-206, are abnormally expressed in certain malignancies and that they play vital roles in tumor initiation, progression, and metastasis. Wei et al. [25] performed a comprehensive profiling of all differentially expressed miRNAs in a total of 519 BC tissue samples. Regarding the clinical value of miR-1 in BC, previous studies have revealed that the expression of miR-1 is aberrant in BC cells. Song et al. [39] identified a group of 51 differentially expressed miRNAs, including miR-1, that may be involved in the initiation of BC and have the potential to serve as biomarkers, and miR-1 was one of the top dysregulated miRNAs verified by RT-PCR analysis. In this study, we first collected existing data from the GEO and ArrayExpress databases, TCGA, and the broader literature. We then performed a more comprehensive analysis of miR-1, taking full advantage of the meta-analysis. Thus far, however, no studies have assessed the diagnostic implications of miR-1 in BC. Consequently, we also examined the diagnostic capacity of miR-1 in BC. The expression of miR-1 in BC was examined by SROC curve analysis. The AUC of SROC analysis suggested that miR-1 may act as a prospective diagnostic indicator of BC. Hence, our results firmly validate the downregulation of miR-1 in BC and indicate that miR-1 has a satisfactory diagnostic value, especially in the early diagnosis of BC.

In recent years, studies of miR-1 in BC have focused on the underlying molecular mechanism while further confirming miR-1 as an effective tumor suppressor. Yoshino et al. [27] found that miR-1 and miR-133a were remarkably downregulated in BC, along with several additional miRNAs, through the targeting of TAGLN2. Yoshino et al. [40] showed that miR-1 restoration induced cell apoptosis through direct inhibition of SRSF9 in BC. Moreover, Yamasaki et al. [41] demonstrated that the expression of miRNA-1 and miR-133a is markedly decreased in BC cells, and their mechanistic exploration showed that silencing of PTMA and PNP greatly repressed cell proliferation and invasion and induced apoptosis. Zhao et al. [42] constructed a recombinant adenovirus with TRAIL expression modulated by miRNA response elements of miR-1, miR-133, and miR-218, namely, Ad-TRAIL-MRE-1-133-218, and demonstrat-

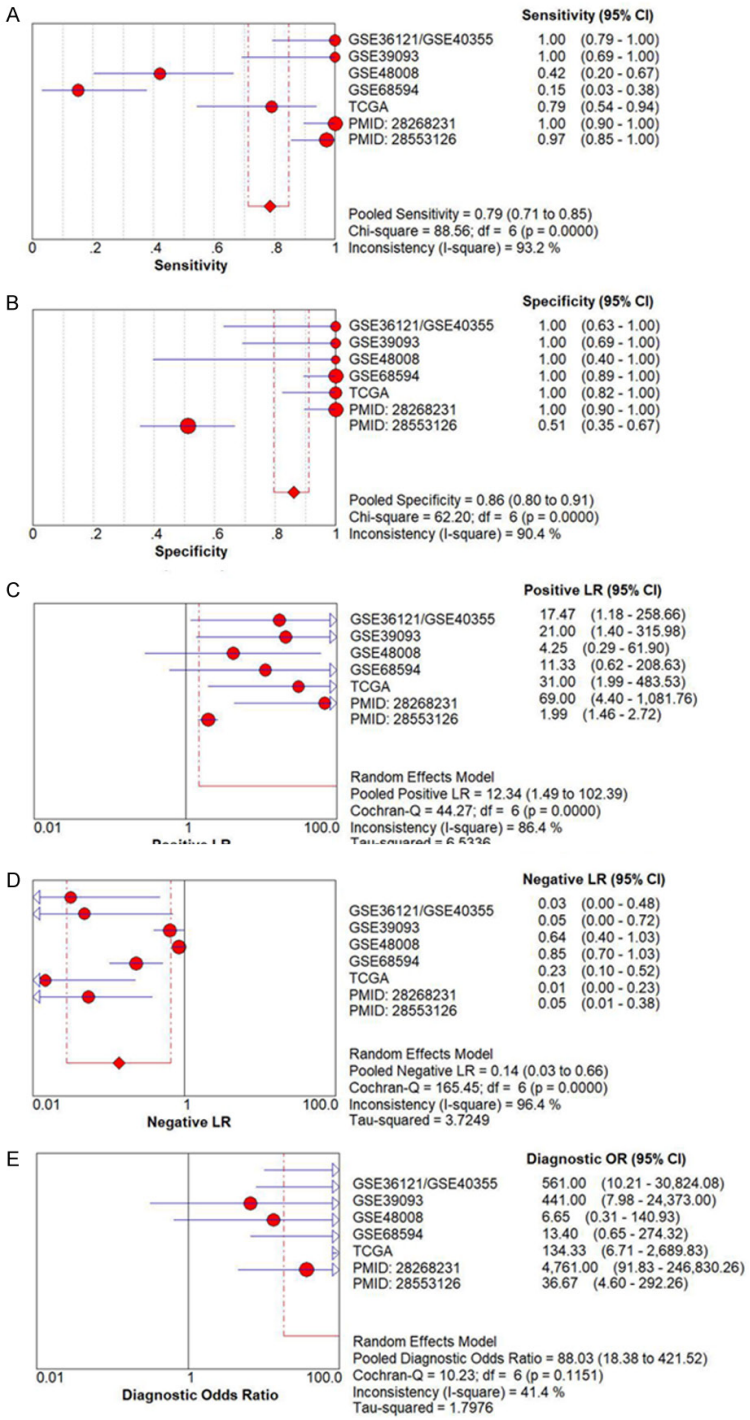

Figure 10. Forest plots of the diagnostic value of miR-1 in bladder cancer. A: Sensitivity; B: Specificity; C: Positive likelihood ratio; D: Negative likelihood ratio; E: Odds ratio.

ed that it effectively blocked the growth of BCs in which miR-1 assisted the gene therapy approach. Wang et al. [43] found that miR-1-3p inhibited the proliferation and invasion of BC cells by suppressing CCL2 expression. Recently, Shang et al. [28] confirmed that miR-1-3p overwhelmed the proliferation, invasion, and migration of BC cells.

In the current study, we also performed bioinformatics analysis to explore the underlying mechanism based on the promising target genes we identified, characterizing miR-1 at a deep level as well as fi-rmly demonstrating its clinical value. The powerful combination of 12 bioinformatics tools for prediction maximized the trustworthiness of the results for miR-1-target-gene prediction. We also mapped a possible principal framework for further investigation of the molecular mechanisms.

According to the results outlined above, several target genes of miR-1 in BC, including TAGLN2, SRSF9, PTMA, PNP, TRAIL, and CCL2, were identified. Regarding signaling pathways associated with miR-1 in BC, Wei et al. [39] reported that most of the relevant signaling pathways involving aberrantly expressed miRNAs were related mainly to Wnt signaling, insulin/IGF, PI3 kinase, and FGF signaling pathways, among others. This study identified a total of 10 bio-pathways: FOXA2/HNF4A, FOXA2/TAT, FOXA2/CDX2, HFN4A/TAT, HNF4A/CDX2, IFIT1/IFIT2, KLK2/HIST1H2AC, PTMA/SLC6A7, KCNJ10/KCNJ16, and ELAVL2/SRSF9. These highly connected targets are involved in important biological processes and molecular functions, such as FOXA2 and HNF4A. David 6.8 analysis showed that the FOXA2/HNF4A signaling pathway connection could be explained by a common overlap, including in maturity onset diabetes of the young (P = 0.087) and sequence-specific DNA binding (P = 0.063). Additionally, in KCNJ10/

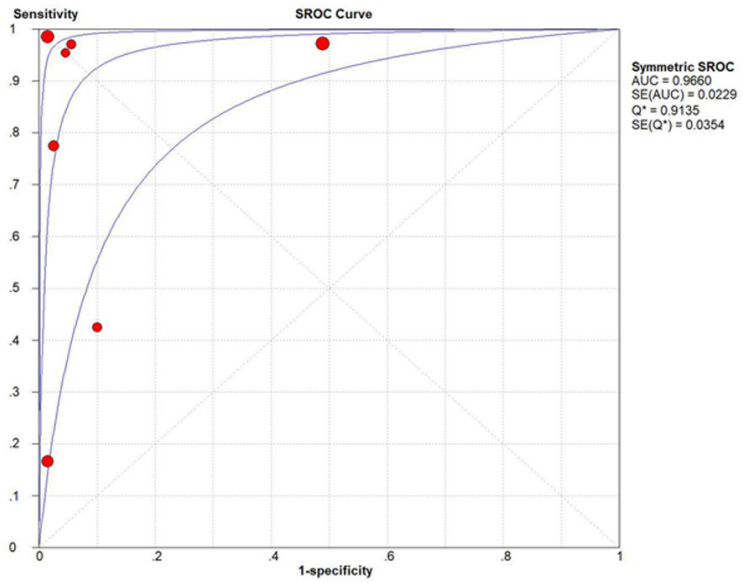

Figure 11. Summarized receiver operating characteristic (SROC) curve.

KCNJ16 signaling, KCNJ10 and KCNJ16 have common components, including regulation of ion transmembrane transport (P = 0.046), potassium ion import (P = 0.083), basolateral plasma membrane (P = 0.093), G-protein-activated inward-rectifier potassium channel activity (P = 0.029), and inward-rectifier potassium channel activity (P = 0.057). These signaling pathways may have a vital regulatory function in proliferation, apoptosis, metabolism, migration, and the invasion of BC; however, the details of the relationships between these signaling pathways and BC need further exploration.

Our study identified 55 genes as promising target genes of miR-1, among which 14 hub genes were ultimately highlighted: TAT, FOXA2, CDX2, HNF4A, IFIT2, IFIT1, HIST1H2AC, KLK2, PTMA, SLC6A7, KCNJ10, KCNJ16, ELAVL2, and SRSF9. These are highly likely to be key target genes of miR-1 in BC. We further mined additional details regarding these target genes, as follows.

Tyrosine aminotransferase (TAT) encodes a mitochondrial tyrosine aminotransferase in the liver and catalyzes the conversion of L-tyrosine into p-hydroxyphenylpyruvate [44]. However, no relationship between TAT and miR-1 in BC has been reported, and thus, further studies are required.

Forkhead box A2 (FOXA2) encodes a member of the forkhead class of DNA-binding proteins. On the basis of experimental and clinical data, it was identified as a biomarker of a transient urothelial progenitor cell population during bladder development. Yamashita et al. [45] concluded that the FOXA protein family consists of crucial controllers of embryonic bladder development and patterning. Reports have also suggested that FOXA2 might be involved in the progression of BC. However, further studies are required to confirm the extent and underlying mechanisms by which FOXA2 might be directly involved in the tumorigenesis of BC, BC treatment responses, and its interaction with miR-1.

Caudal type homeobox 2 (CDX2), a member of the caudal-related homeobox transcription factor gene family, is a main controller of intestine-specific genes and is closely related to cell growth. Kumari et al. [46] showed that the protein expression of CDX2 was reduced in BC tumor cells. Nath et al. [47] indicated that CDX2 can be used to discriminate adenocarcinoma of the bladder from adenocarcinoma of the colon. In summary, CDX2 may play an essential role in BC diagnosis. More work is required to uncover its precise interaction with miR-1 in BC.

Hepatocyte nuclear factor 4 alpha (HNF4A) is a member of a nuclear receptor subfamily that controls genetic transcription of a varied group of genes involved in the synthesis of blood coagulation factors [47]. However, the function of HNF4A in BC has not previously been reported. Further studies are therefore urgently needed.

Both interferon-induced protein with tetratricopeptide repeats 1 (IFIT1) and interferon-induced protein with tetratricopeptide repeats 2 (IFIT2) belong to a family of interferon-induced proteins with tetratricopeptide repeats (IFITs). Studies have found that IFIT proteins constrain virus replication by binding and modifying the functions of cellular and viral proteins and RNAs [48]. IFIT1 may typically exist as monomers, whose shape is similar to a clamp, and have a positively charged pocket responsible

Catalog	Term	Count	%	PValue	Genes
GOTERM_BP_DIRECT	GO:0001568~blood vessel development	3	5.45455	0.00616	DLX3, CDX2, STRA6
	GO:0008344~adult locomotory behavior	3	5.45455	0.0113	FOXA2, FGF12, NTSR1
	GO:0043129~surfactant homeostasis	2	3.63636	0.02451	LPCAT1, ABCA12
	GO:0003254~regulation of membrane depolarization	2	3.63636	0.02753	FGF12, NTSR1
	GO:0050905~neuromuscular process	2	3.63636	0.04548	STRA6, FGF12
	GO:0034765~regulation of ion transmembrane transport	3	5.45455	0.04635	KCNJ16, CALHM1, KCNJ10
	GO:0010107~potassium ion import	2	3.63636	0.08324	KCNJ16, KCNJ10
GOTERM_CC_DIRECT	GO:0005576~extracellular region	9	16.3636	0.08452	LYG2, KLK2, PSORS1C2, CRP, FGF11, FBN2, COL11A1, PNP, BMP8A
	GO:0016323~basolateral plasma membrane	3	5.45455	0.09339	KCNJ16, KCNJ10, SLC10A1
GOTERM_MF_DIRECT	GO:0015467~G-protein activated inward rectifier potassium channel activity	2	3.63636	0.02866	KCNJ16, KCNJ10
	GO:0005242~inward rectifier potassium channel activity	2	3.63636	0.05651	KCNJ16, KCNJ10
	GO:0043565~sequence-specific DNA binding	5	9.09091	0.06287	DLX3, CDX2, FOXA2, HNF4A, SHOX
	GO:0008083~growth factor activity	3	5.45455	0.08033	FGF11, FGF12, BMP8A
KEGG_PATHWAY	hsa04950:Maturity onset diabetes of the young	2	3.63636	0.08664	FOXA2, HNF4A

Figure 12. Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

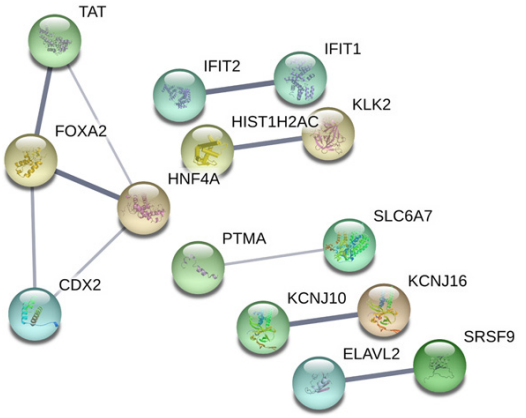

Figure 13. Protein-protein interaction (PPI) network of promising target genes of miR-1 in bladder cancer.

for RNA binding [49]. IFIT2, in contrast, exists as a homodimer, interconnected by intertwined or "swapped" domains. The positively charged cavity in IFIT2 is larger and could develop into a channel [50]. To date, although no study has demonstrated their roles in BC and the relationship with miR-1, future studies may reveal new information.

Histone cluster 1 H2A family member c (HIST1H2AC) is a member of a group of isoforms of histone H2A. Singh et al. [51] reported that variations in the abundance of H2A isoforms are related to the growth and tumorigenicity of BC cells. Decreased expression of the HIST1H2AC locus causes enlarged rates of cell-number increase and tumorigenicity. Singh et al. specified that replication-dependent histone isoforms can have distinct cellular functions and that modulation of these isoforms may play a pivotal role in carcinogenesis. Thus, it is likely that future research will discover a relationship between HIST1H2AC and miR-1 in BC.

Kallikrein-related peptidase 2 (KLK2) encodes a member of the granular kallikrein protein family, which is overexpressed in prostate tumor cells and may serve as a prognostic biomarker for prostate cancer [52]. Our study is the first to identify a potential relationship between KLK2 and miR-1 in BC. Further studies are urgently needed.

Prothymosin-alpha (PTMA) expression was found to be increased in human BC tissues compared to matching paracancerous bladder tissue. The distribution of PTMA expression was altered in high-grade cancers. Yamasaki et al. [41] found that PTMA and purine nucleoside phosphorylase (PNP) are directly modulated by miR-1. PTMA plays an important role in BC. Thus, the clinical implications of PTMA expression in BC are worthy of further inquiry.

Solute carrier family 6 member 7 (SLC6A7) is a member of the gamma-aminobutyric acid (GABA) neurotransmitter gene family and encodes a high-affinity mammalian L-proline transporter protein in the brain [53]. However, no studies have reported a relationship between miR-1 and SLC6A7 in BC, necessitating further research.

Potassium voltage-gated channel subfamily J member 10 (KCNJ10) encodes a member of the inward-rectifier type potassium channel family, which is characterized by a superior trend toward allowing potassium to flow into, rather than out of, a cell. KCNJ10 may be responsible for the potassium buffering action of glial cells. KCNJ16 encodes an integral membrane protein and inward-rectifier type potassium channel, which tends to permit potassium to flow into rather than out of a cell, and it may play a role in fluid and pH regulation [54, 55]. Further studies are needed to explore the relationship between KCNJ10, KCNJ16, and miR-1 in BC.

ELAV-like RNA binding protein 2 (ELAVL2) encodes a neural-specific RNA-binding protein that is recognized to bind to several 3'UTRs [56]. The relationship between ELAVL2 and miR-1 or BC also requires further research.

Serine and arginine rich splicing factor 9 (SRSF9) encodes a member of the serine/arginine (SR)-rich family of pre-mRNA splicing factors, which are essential for mRNA splicing. Studies have demonstrated the involvement of SR proteins in mRNA export from the nucleus and the process of translation. Yoshino et al. [40] showed that miR-1 could enhance apoptosis via direct suppression of SRSF9 in BC. The documentation of molecular mechanisms between miR-1 and SR proteins, including new apoptosis pathways and their epigenetic modulations, may provide novel tactics for BC treatment [40].

In summary, the genes TAT, FOXA2, CDX2, HNF4A, IFIT2, IFIT1, HIST1H2AC, KLK2, PTMA, SLC6A7, KCNJ10, KCNJ16, ELAVL2, and SRSF9 have a high likelihood of being target genes of miR-1. The current study shows that they contribute to the metabolism and biosynthesis of BC, which is associated with their signaling pathways. In view of all the relevant studies, we conclude that more studies are desirable to confirm the association between miR-1 and its theorized target genes in BC.

Our study has several limitations. First, the role of miR-1 in determining prognosis remains unverified and requires additional analysis. Second, the underlying mechanisms of the PPI network remain unclear; they require further exploration.

Conclusions

This study aggregates data from multiple resources (GEO, ArrayExpress, TCGA, and several publications) and confirms that miR-1 acts as a tumor-suppressive miRNA and exerts a vital effect on the diagnosis of BC. MiR-1 may execute its suppressive role in BC by modulating a network of target genes (including TAT, FOXA2, CDX2, HNF4A, IFIT2, IFIT1, HIST1H2AC, KLK2, PTMA, SLC6A7, KCNJ10, KCNJ16, ELAVL2, and SRSF9) through specific signaling pathways. Further studies are required to determine the potential mechanisms of miR-1-regulated networks in BC.

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Disclosure of conflict of interest

None.

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